CALIFORNIA DEPARTMENT OF PESTICIDE REGULATION
TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

ACROLEIN

Chemical Code # 0003, Tolerance # 50032
SB 950 # 004

August 7, 1986
Revised 12/12/86, 2/8/88, 11/23/88, 11/7/90, 8/18/94

I. DATA GAP STATUS

Chronic toxicity, rat:	Data gap, inadequate study, no adverse effect indicated
Chronic toxicity, dog:	No data gap, possible adverse effect
Oncogenicity, rat:	No data gap, possible adverse effect
Oncogenicity, mouse:	Data gap, inadequate study, no adverse effect indicated
Reproduction, rat:	No data gap, possible adverse effect
Teratology, rat:	No data gap, no adverse effect
Teratology, rabbit:	No data gap, no adverse effect

Teratology, mouse:	Inadequate study, possible adverse effect indicated
Gene mutation:	No data gap, possible adverse effect
Chromosome effects:	No data gap, no adverse effect[1]
DNA damage:	No data gap, no adverse effect[1]
Neurotoxicity:	Not required at this time

[1]Studies in the open literature but not on file at DPR indicate a possible adverse effect under these categories.

Toxicology one-liners are attached.
** indicates an acceptable study.
Bold face indicates a possible adverse effect.
These pages contain summaries only. Individual worksheets should be reviewed as they may contain additional effects.

All record numbers through 128724 were examined.

File name: **T940818**
Revised by: Stephen J. Rinkus on 8/18/94

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

CHRONIC TOXICITY, RAT

Note: record 085062, a combined chronic toxicity/oncogenicity study, is considered acceptable as an oncogenicity study but is considered unacceptable and not upgradable as a chronic toxicity study. The one-liner for record 085062 appears under the heading "ONCOGENICITY, RAT." (Rinkus, 8/18/94).

CHRONIC TOXICITY, DOG

****50032-026 073320** "Acrolein--Chronic (12 Month) Oral Toxicity Study in the Dog," (Long, J.E., Tegeris Laboratories Inc., Project I.D. no. TL #85016; 10/23/87). Acrolein, purity of $\geq$ 94%, was administered by gelatin capsule at concentrations of 0, 0.1, 0.5 or 2.0 mg/kg/day to 6 beagle dogs/sex/group for 12 months. Dose levels were based on a range-finding study, which was includ-ed in the report. The only effects identified in the first review (9/11/90) as related to treatments were: vomiting in the mid and high-dose groups (both sexes), with the frequency being greater in the high-dose groups; and de-creases in the serum levels for calcium and albumin (consequently total pro-tein, too) for the high-dose male and female groups, with both reductions being seen at each of the 3-month testing intervals. When first reviewed, this study was considered unacceptable, but upgradable upon submission of the ophthalmology raw data, a complete list of protocol deviations and resolution of matters concerning the route of exposure, preparation of dosing solutions, controlling of mites, frequency of dosing per week, and assaying for serum gamma-glutamyl transpeptidase. The Registrant responded by submitting records 097215 and 128724. These records are discussed in worksheet W073320.S01. A decrease in the activated partial thromboplastin time (hypercoagulation) is now being identified as a possible adverse effect (**NOAEL = 0.1 mg/kg**),

for reasons discussed in worksheet W073320.S01. This study is now considered **ACCEPTABLE**. (Rinkus, 5/13/94).

006 042955. Protocol reviewed.

50032-036 097215 This record uses a question-and-answer format to address is-sues raised in worksheet W073320.821. **Supplemental information**. (Rinkus, 5/18/94).

50032-049 128724 This record contains 11 protocol deviations for record 073320. **Supplemental information**. (Rinkus, 5/18/94).

ONCOGENICITY, RAT

****50032-028 085062** "24-Month Chronic Toxicity and Oncogenicity Study in the Rat with Acrolein," (Long, J.E. & Johnson, J.A., Tegeris Laboratories, Inc., Laboratory Project No. TL 85047; 9/6/89). Acrolein, purity N96%, was admin-istered by gavage at the nominal concentrations of 0 (water), 0.05, 0.5 and 2.5 mg/kg to 70-75 Sprague-Dawley rats/sex/group, for 102 weeks. Analytical testing of dosing solutions was done only 0-4 times/month for the first 18 months of the study. Dose levels were based on a short (6-week) pilot study, which was also included in the report. At 13 weeks and at 1 y, 5 high-dose rats (both sexes) and 10 rats/sex/group were sacrificed, respectively. Also, gavaging-induced deaths and loss of tissues due to autolysis effectively re-duced the number of rats/sex/treatment level, depending on the tissue in ques-tion. There were no obviously treatment-related effects on the following: sur-vival, bodyweights, organ weights, clinical signs, serum chemistry, hematolo-gy, urinalysis, and gross findings. The ophthalmology portion of this study was inadequate for interpretation. Two possible histological findings were increased incidences of hepatocellular adenomas and acinar-cell adenomas of the pancreas in the males; both tumor types exhibited dose responses and max-imum percentages affected that were clearly greater than the values observed in the supplied historical control data. No histological findings

were noted in the obvious target organ, the forestomach. This study was considered unacceptable when first reviewed (Rinkus, 8/13/90); and upgrading would require the submission of supplemental information that addressed the many concerns that DPR MT had about the study. The Registrant responded by submitting re-cords 097781, 124586 and 126430. These records are discussed in worksheet W085062.S01. Based on these submissions, record 085062 is considered margin-ally **ACCEPTABLE** only as an oncogenicity study. Hepatocellular adenomas in males have been dropped as an adverse-effect finding but pancreatic acinar cell tumors (adenoma and carcinoma) remain as adverse effects, with a **LOAEL of 0.05 mg/kg**. Record 085062 is **NOT ACCEPTABLE** and **NOT UPGRADABLE** as a chronic-toxicity study (inadequate hematology). (Rinkus, 3/18/94).

006 042957. Protocol reviewed. Study due Jan., 1989.

011 059502. Status at 30 weeks in terms of survivors. Doses of 0, 0.05, 0.5, and 2.5 mg/kg are being given by oral gavage. Doses were selected based on a 6-week range-finding study. Document is dated December 23, 1986. No review sheet. (Gee 2/5/88)

50032-044 097781 This record uses a question-and-answer format to address is-sues raised in worksheet W085062.835. **Supplemental information**. (Rinkus, 5/18/94).

50032-047 124586 This record is the pilot study on which the selection of the high dose for the rat reproduction study (record 092721) was based. **Supple-mental information.** (Rinkus, 5/18/94).

50032-048 126430 This record contains three mailings of explanatory informa-tion regarding the statistical analyses contained in record 097881 for hepatic and pancreatic tumors in males. **Supplemental information.** (Rinkus, 5/18/94).

ONCOGENICITY, MOUSE

50032-029 090136, "18-Month Oncogenicity Study in the Mouse with Acrolein," (Long, J.E. & Johnson, J.A., Tegeris Laboratories, Inc., Laboratory Project No. TL 86057; 10/16/89). Acrolein, purity N96%, was administered by gavage at concentrations of 0 (water), 0.5, 2.0, and 4.5 mg/kg to CD-1 Swiss Albino mice for 18 months. Dose levels were based on a short (6 week) pilot study, which was also included in the report. Although the original study design called for 70-75 mice/sex/group, gavaging-induced deaths during the first few weeks of the study reduced the "effective number" of mice/sex/group to 37-67; and autolysis prevented the histological examination of some tissues, thereby re-ducing further the "effective number" for some organs. Excluding the gavaging-induced deaths occurring before week 3, there was no evidence that survival was affected in this study. Also, there was no evidence of a treatment-relat-ed effect on the following: bodyweights; clinical signs; wet organ weights (terminal sacrifice) for the brain, liver, kidneys, and testes; WBC differen-tials; and ophthalmology. Neither gross nor microscopic examinations identi-fied any treatment-related effects, including no effects on the obvious target organ for this direct-acting agent, the forestomach. **NOEL > 4.5 mg/kg.** This study was considered **UNACCEPTABLE** when first reviewed (Kishiyama and Rinkus, 7/5/90) and upgrading would require the submission of supplemental informa-tion that would address the following: whether the highest dose tested was sufficiently close to the maximum tolerated dose; mice brought late into the study; the choice of the oral route of exposure over inhalation; amendments and deviations to the protocol; and discontinued QA inspections. The Regis-trant responded by submitting record 097780 and its contents are discussed in worksheet W090136.S01. This study is considered **UNACCEPTABLE** and **NOT UPGRAD-ABLE (MTD not tested, poor study conduct, data for a parallel study not sub-mitted)**. (Rinkus, 9/7/93).

006 042959. Protocol reviewed. Study due Jan., 1989.

011 059216. Status report at week 5 for mouse study with survivors. Doses of 0, 0.5, 2.0, and 4.5 mg/kg are being given by oral gavage. A cover letter, dated July 10, 1987, discusses the problems Tegeris Laboratories is having with the dosing, resulting in numerous deaths. Additional mice will be added to the original 70/sex/group at the same doses so that an

adequate number should be available at termination of the study. Status report is dated December 23, 1986. No review sheet. (Gee 2/5/88). Inspection of the data for the full study (record 090136) indicates that the water-gavaged mice also were dying as much as the acrolein-gavaged mice were. Therefore, the dying in this study appears to have been due to the gavaging technique and not the fact that three of the four dosing solutions contained acrolein. (Rinkus, 9/28/90).

50032-043 097880 This record uses a question-and-answer format to address is-sues raised in worksheet W090136.832. **Supplemental information.** (Rinkus, 5/18/94).

REPRODUCTION

007-8 50058-9, "Two Generation Reproductive Study of Acrolein in Albino Rats", (Bioassay Systems Corporation, 12-7-84). Acrolein by gavage at 0, 4.0, 5.4 and 7.2 **mg**/kg/day. Reproductive NOEL > 7.2 mg/kg. Chronic toxicity NOEL < 4.0 mg/kg (decreased body weight F1 males, stomach lesions F0 and F1). UNACCEPTABLE; can not be upgraded (limited histopathology and necropsy, poor study performance, gestation period ranged from 11 to 27 days). No adverse effect. (Parker 12/16/86). Rebuttal by Baker Performance Chemicals and supplemental data (Record 067445) led to no change in evaluation. (Davis 10/5/88).

021 067445, "Report Amendment: Two Generation Reproductive Study of Acrolein in Albino Rats." Supplemental material (revisions and corrections for the report; dosing solution analyses; dosing records; breeding observations) in support of the rebuttal in volume 021, part 1. (Davis 10/5/88).

025 072525. Audit of the study prepared at Argus Research Laboratories. No worksheet. (Gee, 2/16/89).

****50032-035 092721** "Reproductive Effects of Acrolein Administered Orally via Gavage to Crl:CD*(SD)BR Rats for Two Generations, with One Litter per Gener-ation," (Alan M. Hoberman, Argus Research Laboratories, Inc., Horsham, PA; Re-port # 603-003, 4/12/91). Acrolein, >96% purity, was administered by gavage once daily at 0 (water), 1, 3, and 6 mg/kg, to 30 Crl:CD*(SD)BR rats/sex/dose in the F0 generation and to 40 rats/sex/dose in the F1 generation. F0 rats and F1 rats, derived from F1a litters, were exposed for N10 weeks before their single mating trials and were exposed for a total of 93-149 daily dosings be-fore they were sacrificed. No effects on the mating, fertility, or gestation indices were observed in either mating trial. Forestomach hyperplasia and (or) hyperkeratosis was seen at ≥ 80% incidence in the 6 mg/kg groups (both sexes, both generations); also, an incipient effect was present at the 3 mg/kg level (females only, both generations). The incidences of mortalities and respira-tory complications (e.g., rales, gasping) were increased significantly in the 6 mg/kg groups (both sexes, both generations). The composite picture that emerges from considering these mortalities and their respective clinical, nec-ropsy and histology data is that aspiration of the dosing solution was common-place, it resulted in lung damage, and in many instances this resulted in deaths. That is, these aspiration-associated lung effects and deaths may not represent a systemic or gastrointestinal effect by acrolein; rather, they are directly dependent on the use of gavage to accomplish oral dosing with a known pulmonary toxicant. **Parental NOAEL = 1 mg/kg (hyperplasia/hyperkeratosis).** The only progeny effect was a significant reduction in pup bodyweights, starting on lactation day 7 for the 6 mg/kg group (F1a offspring only). For the F1 males (but not the F1 females) derived from the F1a 6 mg/kg pups, this reduc-tion in bodyweight (relative to the controls) persisted till their sacrifice at the end of the study. **Progeny NOAEL = 3 mg/kg (reduced F1a pup bodyweights during lactation).** This study is considered **ACCEPTABLE**. (Rinkus, 4/12/94).

50032-047 124586 This record is the pilot study on which the selection of the high dose for the rat reproduction study (record 092721) was based. **Supple-mental information.** (Rinkus, 5/18/94).

50032-046 124351 This record is the protocol for the rat reproduction study (record 092721). **Supplemental information.** (Rinkus, 5/18/94).

50032-046 124343 This record is the protocol for the pilot rat reproduction study (record 124586). **Supplemental information.** (Rinkus, 5/18/94).

TERATOLOGY, RAT

**004 021617 "Teratology study of Acrolein in Rats", (Bioassay Systems Corp., 11-12-82, Project No. 10258). Acrolein, Batch 6151, 96.48%; analysis in 063285; given by oral gavage to Sprague-Dawley CD rats, days 7-19 (day of mating = day 1) at 0, 3.6, 6.0, or 10 mg/kg/day; maternal NOEL = 3.6 mg/kg/day (decreased weight gain, increased mortality and clinical signs), developmental NOEL = 6.0 mg/kg/day (decreased fetal weight and increased skeletal variants). Initially reviewed by J. Christopher, 3/27/85. Rereviewed by J. Parker, 8/6/86, as not showing an adverse developmental effect. Submission of supple-mental data in 063285 provides sufficient additional information to upgrade the study to ACCEPTABLE status with minor deficiencies. No adverse effect. (Gee, 2/5/88, with Parker).

EPA 1-liner: Minimum. Maternal NOEL = 3.6 mg/kg (decreased body weight gain and increased mortality), fetotoxic NOEL = 6.0 mg/kg (decreased fetal weights, delayed ossifications), teratogenic NOEL = 6.0 mg/kg (fetal runts).

014 063285 "Report Amendment--Teratology Study of Acrolein in Rats," (MacRill, G.E.; Baker Performance Chemicals, Inc.; "revised" 8/14/87). This is also en-titled "Appendix F" (to record 021617). It contains supplementary data to ad-dress the concerns raised by the then CDFA toxicologists that reviewed record 021617. These concerns are summarized in a question-and-answer format in a four-page letter dated October 23, 1987 from the Registrant to then CDFA that accompanied this submission; this letter (no record number) appears in the front of 50032-014. **Supplementary information.** (Rinkus, 5/18/94).

50032-003 920144 This record is a two-page summary of the results for the rat teratogenicity study (record 021617) and the mouse teratogenicity study (record 021618). A letter dated

November 2, 1982 from the Registrant to then CDFA accompanied this submission; this letter (no record number) appears in 50032-003 just before record 920144. **Supplemental information.** (Rinkus, 5/18/94).

013 061605, "Comparison of the Mutagenicity and Teratogenicity of Cyclophosphamide and Its Active Metabolites, 4-Hydroxycyclophosphamide, Phosporamide Mustard, and Acrolein", (McGill University, Montreal, Cancer Research (1982) 42, 3016-3021). Acrolein (a metabolite of cyclophosphamide) was one of the 4 chemicals given to rats by an intra-amniotic injection to fetuses in one uterine horn on day 13 of gestation. At 100 ug/fetus, 98% of fetuses were dead or resorbed, at 10 ug/fetus, 100% of fetuses were dead or resorbed. At 1 ug/fetus, 85.7% of the fetuses had malformations very similar to those caused by cyclophosphamide (edema, hydrocephaly, open eyes, cleft palate, omphalocele and forelimb, hindlimb and tail defects.) At 0.1 ug, 0.002 umol/fetus, acrolein had no effect on resorption or malformation rate compared with saline control. Fetal weight was decreased at both 0.1 and 1 ug/fetus. The chemicals were also tested in an Ames assay with strain TA1535 of S. typhimurium at 0.001 to 50 ug/plate. Acrolein was very bacteriotoxic, not mutagenic without S9 and very weakly mutagenic with S9 activation. Supplementary. Not a guideline type study. Possible adverse developmental effect in fetuses and weakly mutagenic in bacteria. (Shimer 11/13/87 and Gee 2/2/88).

TERATOLOGY, RABBIT

** 015 063594, "Developmental Toxicity (Embryo/Fetal Toxicity and Teratogenic Potential) Study of Acrolein Administered Orally (Stomach Tube) to New Zealand White Rabbits", (Argus Research Laboratories, Inc., Report no. 603-001, 5-20-87). Acrolein, 96.15% was administered to inseminated New Zealand White rabbits on days 7-19 of gestation at 0, 0.1, 0.75, and 2 mg/kg/day, 20/group. Maternal NOEL = 0.75 mg/kg/day (initial reduction of body weight gain; Developmental NOEL = 0.75 mg/kg/day (increase in number of resorptions not statistically significant). No adverse effect. ACCEPTABLE. (Shimer 1/12/88 and Gee 2/3/88).

TERATOLOGY, MOUSE

003 021618, "Teratology Study of Acrolein in Mice", (Bioassay Systems Corp., 9-1-82). Acrolein, Batch 6104, 96%, by oral gavage on days 7 - 17 of gestation, at 0, 4.0, 6.3, and 10 mg/kg/day. Developmental NOEL less than 4.0 (Resorptions at 10, increase in structural changes at all dose levels). Maternal NOEL = 6.3 mg/kg (decreased weight gain). UNACCEPTABLE, not upgradeable and **possible adverse effect indicated**. (Christopher 3-28-85, Parker 8-6-86).

EPA 1-liner: Supplementary. Maternal NOEL <= 4 mg/kg (LDT) (decreased body weight gain); fetotoxic NOEL <= 4 mg/kg (generalized delayed ossi-fication); teratogenic NOEL, not established: possible cleft plate at 6.3 and 10 mg/kg. Conclusion could not be reached due to lack of critical data in report.

GENE MUTATION

Summary. The data requirement for gene mutation studies (842) is considered filled, with an adverse effect indicated. The results from bacterial testing included positive results (records 061604 & 061607), borderline results (re-cords 061603, 061605, 061606 &061609), and negative results (records 061602 & 061603). The two studies indicating a reproducible mutagenic effect also have provided a rationale for why other studies may have failed to demonstrate mu-tagenicity and this has persuaded CDFA to treat acrolein as a potential muta-gen. The fact that acrolein is very reactive with sulfhydryl groups makes it very cytotoxic; as a result, "nonstandard" test procedures are needed to dem-onstrate mutagenicity. In record 061607, the new Salmonella tester strain TA104 was used with a liquid preincubation method of exposure; also, it was found that the presence of the plasmid pKM101 and deletion of the uvrB gene facilitated the detection of acrolein and that adding glutathione after the liquid preincubation period reduced the cytotoxicity but not the mutagenicity of acrolein. In record 061604, TA100 was used with a liquid preincubation method; afterwards, the cells were isolated

by centrifugation and resuspended into fresh phosphate buffer for plating. Elsewhere one of the authors of record 061604 has reported that the liquid preincubation method using an increased cell density is necessary for demonstrating the mutagenicity of acrolein (Naunyn-Schmied. Arch. Pharmacol. 316 (Supplement), Abstract 54, 1981); these same conditions also are critical for detecting 3-methylacrolein (Environ. Molec. Mutagen. 14: 146-148, 1989). (Rinkus, 9/28/90).

50032-027 075135 "CHO/HGPRT Mutation Assay with Confirmation," (J.W. Har-bell, Microbiological Associates Inc., Laboratory Study No. T8403.332001; 5/25/89). Acrolein, 96% purity, was tested as dimethylsulfoxide solutions for mutagenicity at the HGPRT locus (6-thioguanine resistance) using Chinese ham-ster ovary K_1-BH_4 cells. Test concentrations ranged from 0.0002 to 0.008 µl/ml and testing was done in the absence and presence of a Aroclor-induced rat liver S-9 activation system; in both cases, cells were exposed to acrolein for 5 h. Testing also involved the routine use of two negative controls (no treatment & DMSO at 1% v/v) and two positive controls (ethyl methanesulfonate and benzo(a)pyrene), which in all cases gave satisfactory results. Three tri-als, each involving testing up to concentrations that produced significant cy-totoxicity, were conducted. In the first two trials, testing in the absence and presence of a S-9 mix was done, while in the third trial, no metabolic activation system was used. Slightly increased mutant frequencies were ob-served with concentrations of 0.0060 µl/ml in the presence of S-9 in the first trial and 0.0008 µl/ml in the absence of S-9 in the second trial. However, in both cases, these increased mutant frequencies were not observed again in re-testing that either included the suspect concentration or testing up to high cytotoxicity. Therefore, **no reproducible mutagenicity was demonstrated. ACCEPTABLE. (Kishiyama & Rinkus, 9/21/90).

006 042949, "In vitro Gene Mutation Assay (HGPRT Locus) in Cultured Chinese Hamster Ovary (CHO) Cells on Acrolein," (Bioassay System Corp., 4-28-82). Acrolein (>95%) at 0, 0.04, 0.06, 0.08, 0.1, 0.2, and 0.3 ug/ml with S9 rat liver activation, and 0, 0.1, 0.2, 0.3, 0.4, and 0.5 ug/ml - S9 on Chinese Hamster cells (CHO); HGPRT system; single trial, duplicate cultures; No evidence of increased mutation frequency; UNACCEPTABLE (No repeat trial). (Gee 8-4-86).

** 013 061602, "Salmonella Liquid Suspension Mutant Fraction Assay on Acrolein", (Bioassay Systems, Project no. 10258, 12-30-80). Acrolein, > 99%, was assayed with Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538 at concentrations of 1, 3, 10, 20, or 40 ug/ml in DMSO with and without S9 activation, duplicate samples, repeat trials with three strains. Suspensions were plated for viability and revertants. At 40 ug/ml, survival was less than 5%. No increase in revertants was noted. ACCEPTABLE. (Shimer 1-13-88 and Gee 2-1-88).

Note: Although this report conforms with guidelines with minor variations, the negative results are in contrast to the positive findings of other investigators also using Salmonella strains. The overall conclusion for gene mutation is that acrolein is a mutagen. See Summary above. (Gee 2/5/88; Rinkus 9/28/90).

The following one-liners are literature citations which contain gene mutation assays on acrolein.

013 061603, "Mutagenicity of Vinyl Compounds in Salmonella typhimurium", (Frederick Cancer Research Center, in Teratogenesis, Carcinogenesis, and Mutagenesis (1980) 1: 259-267). Eighteen compounds structurally related to vinyl chloride were tested for mutagenicity in five strains of Salmonella typhimurium - TA1535, TA1537, TA1538, TA98 and TA100. All chemicals were greater than 95% pure, activation was provided with rat and hamster S9 mix. Acrolein was mutagenic without activation only at concentrations from 0.02 to 0.07 ul in strain TA98 in the plate incorporation assay. UNACCEPTABLE. No individual plate counts. Publication indicates repeat trials were run for mutagenic compounds but no data for repeat trial. (Shimer 1/13/88 and Gee 2/1/88).

013 061604, "Structure Mutagenicity Relationship in α,β-Unsaturated Carbonylic Compounds and Their Corresponding Allylic Alcohols", (Mutation Research (1982) 93: 305-315). Several compounds were tested with strain TA100 of Salmonella typhimurium for mutagenic activity in a modified suspension assay, incubated for 90 minutes and plated in duplicate. Two independent trials were run. Acrolein, 99.9% was found to be highly mutagenic with 2400 revertants per

µmole reported without activation. It was nonmutagenic and less cytotoxic with S9 activation. Apparently tested at 0 to 0.15 µmoles/2 mls incubation volume. Publication contains the statement that acrolein was negative in the plate incorporation assay. UNACCEPTABLE. One strain, no individual plate counts - data presented in graphic form only. (Shimer 1/13/88 and Gee 2/1/88).

013 061605, "Comparison of the Mutagenicity and Teratogenicity of Cyclophosphamide and Its Active Metabolites, 4-Hydroxycyclophosphamide, Phosphoramide Mustard, and Acrolein", (McGill University, Montreal, Cancer Research (1982) 42: 3016-3021). See under Rat Teratology above for one-liner.

013 061606, "Salmonella Mutagenicity Test Results for 250 Chemicals", (EG&G Mason Research Institute, Environmental Mutagenesis Supplement (1983) 1: 3-142). Four strains of Salmonella, TA1535, TA1537, TA98 and TA100, were used to test 250 coded chemicals, at three different labs - Case Western Reserve, Microbiological Associates and SRI. Male Sprague-Dawley rats and male Syrian hamsters were used for liver activation. Acrolein, practical grade, was 82% and was tested at 0 to 100 ug/plate in triplicate with a repeat trial. The preincubation procedure for 20 minutes was used. The summary table indicates a positive effect (+) with acrolein but the data do not substantiate this conclusion. TA100 shows a less than 2-fold increase in revertant colonies with rat liver activation and less of an increase with hamster activation. There is , however, a concentration-dependent trend. Acrolein was tested to the limit of cytoxicity. UNACCEPTABLE. Data presented as mean $\pm$ SEM, practical grade was used. (Shimer 1/13/88 and Gee 2/2/88).

013 061607, "Naturally Occurring Carbonyl Compounds are Mutagens in Salmonella Tester Strain TA104", (U.C. Berkeley, Mutation Research (1985), 148: 25-34). Acrolein, purity not stated; a new Salmonella tester strain, TA104 (nonsense mutation - TAA - at site of reversion in a single copy), was tested with carbonyl compounds to see how it compared with other strains in assaying for mutagenicity. In the liquid preincubation procedure, 20 minutes with shaking, in duplicate, acrolein was mutagenic in TA104 but not TA102 (no data). The maximum non-toxic concentration was 0.9 umoles with 1080 revertants/umole with TA104. The text contains the

statement that acrolein was much less mutagenic in TA2638 (uvr+) than in TA104. The marked toxicity of acrolein limits its detectability as a mutagen. A reference is made to the formation of adducts by acrolein with deoxyguanosine. UNACCEPTABLE. Data in graphic form only, one strain only. **Possible adverse effect with increase in revertants**. (Shimer, 1/1/3/88 and Gee 2/2/88).

013 061608, "Mutagenic Activity of Major Mammalian Metabolites of Cyclophosphamide Toward Several Genes of Escherichia coli", (National Institute of Environmental Health Sciences, J. of Toxicology and Environmental Health, (1977) 3:637-650). Acrolein, 98%, along with other compounds was tested with E. coli 343/113 for its ability to induce mutations. After treatment for 180 minutes, E. coli was plated on selective medium for detection of back mutations to gal+ and arg+ MTR (from 5-methyltryptophan sensitivity to resistance) forward mutations. Acrolein did not cause an increase in mutation frequency - tested only without activation. It was quite cytotoxic - data inactivation of E. coli are presented. UNACCEPTABLE. No data for mutation frequency, no activation included. (Shimer 1/13/88 and Gee 2/2/88).

013 061609, "Comparison of Alkylation Rates and Mutagenicity of Directly Acting Industrial and Laboratory Chemicals", (Institute of Occupational Health, Finland, Arch. Toxicol. (1980) 46:277-285). The alkylation activity was compared with mutagenicity of industrial and laboratory chemicals to E. coli WP2 uvrA without metabolic activation, incubated for 18 hours, then plated for revertants. Acrolein, analytical grade, no purity stated, was considered as showing "weak" mutagenicity and alkylation activity with 4-(p-nitrobenzyl)-pyridine and deoxyguanosine. The mutagenicity is expressed as the change in reversion frequency X 10(-11)/uM (4) or as % in relation to epichlorohydrin (2) so the significance is difficult to evaluate. UNACCEPTABLE. No activation, no plate counts, no concentrations tested, other missing information. (Shimer 1/19/88 and Gee 2/2/88).

013 061610, "Chemical Mutagenesis Testing in Drosophila. II. Results of 20 Coded Compounds Tested for the National Toxicology Program", (Environmental Mutagensis (1985) 7:87-100). Results are presented from mutagenesis testing in the sex-linked recessive lethal test in

Drosophila of 20 coded compounds. Acrolein, 96.9%, considered negative. In feeding studies, males were placed in vials containing glass filters saturated with the test material in 5% sucrose at 0 or 3000 ppm and exposed for 3 days, then mated. For injection studies, males were mated 24 hours after injection with 0 or 200 ppm (volume not stated). Canton-S wild-type males were each mated to three Basc females to produce three broods over 7 days. Two experiments were run for a total of >5800 total tests. An earlier study is cited in which acrolein reportedly gave positive effects after larval feeding - no data. UNACCEPTABLE. No justifications of the concentrations used, unclear if two trials with both feeding and injection. (Shimer 1/19/88 and Gee 2/2/88).

CHROMOSOME EFFECTS

Note: CDFA is aware of the following reports in the open literature that indicate positive and equivocal effects under this category: the induction of sister-chromatid exchanges in CHO cells (Au et al., Cytogenetics and Cell Genetics 26:108-116, 1980; Galloway et al., Environ. Molec. Mutagen. 10 (Suppl. 10): 1-175, 1987).

** 006 042952, "Effects of Acrolein on the In Vitro Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells", (Bioassay Systems Corp., 5/1/82). Acrolein (>95%) tested 0, 0.01, 0.3, 0.5, and 0.75 µg/ml on CHO cells, +S9, 4 hours; at 0, 0.3, 0.5, and 0.75 µg/ml -S9, for 30 hours. NO evidence for SCE formation; ACCEPTABLE. (Gee 8-4-86)

** 006 042951, "Effects of Acrolein on the In Vitro Induction of Chromosomal Aberations in Chinese Hamster Ovary Cells", (Bioassay Systems Corp., 7/23/82). Acrolein (>95%) tested at 0, 0.4, 0.6, 0.8, 1.0, 1.5, and 2.0 µg/ml +S9 on CHO cells, 2 hours, and 0, 0.2, 0.4, 0.6, 0.8, 1.0, and 2.0 -S9 for 6 hours; single time of harvest, duplicate cultures; No adverse effects reported; ACCEPTABLE with variances (single time of harvest and varying number of cells analyzed.) (Gee 8-4-86).

006 042950, "Effects of Acrolein on the In Vivo Induction of Chromosomal Aberrations in Rat Bone Marrow Cells", (Bioassay Systems Corp., 11/17/82).Acrolein (>95%) given at 0, 1, 2.1, and 4.1 mg/kg in a single i.p. injection to Sprague-Dawley rats, 3 to 5 males/group; sampling times of 6, 12, and 24 hours; No evidence of increased chromosomal abberations; UNACCEPTABLE (males only). (Gee 8-7-86).

DNA DAMAGE

Note: CDFA is aware of the following reports in the open literature that indicate a positive effect under this category: the induction of DNA single-strand breaks, as measured by the alkaline elution assay (Erickson et al., Cancer Research 40: 4216-4220, 1980); and the formation of DNA adducts (Chung et al., Cancer Research 44: 990-995, 1984; Wilson et al., Proceedings of the American Association for Cancer Research 31:95 (Abstract No. 563), 1990).

** 006 042953, "Effect of Acrolein on the Incidence of C3H/10T1/2 transformed cells In Vitro", (Bioassay System Corp., 4/28/82). Acrolein (>95%) tested at 0, 0.04, 0.06, 0.08, and 0.1 ug/ml on mouse fibroblasts (C3H/10T1/2), exposed for 3 days; 20 dishes/concentration; scored at 6-7 weeks; No increased incidence of type II - III foci reported; ACCEPTABLE. (Gee 8-4-86).

NEUROTOXICITY

Not required at this time.

Publications Regarding Acrolein

Anon. (1985) Acrolein. In: IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Volume 36, pp. 133-161. World Health Organization, Lyon, France.

Beauchamp, Jr., et al. (1985) A Critical Review of the Literature on Acrolein Toxicity. CRC Critical Reviews in Toxicology 14: 309-380.

Witz, G. (1989) Biological Interactions of α,β-Unsaturated Aldehydes. Free Radical Biology & Medicine 7: 333-349.

Selley et al. (1990) Effects of Acrolein on Human Platelet Aggregation. Chem.-Biol. Interactions 76:101-109.

Lijinsky, W. and Reuber, M.D. (1987) Chronic Carcinogenesis Studies of Acro-lein and Related Compounds. Toxicology and Industrial Health 3:337-345.

Published Versions of Some of the Studies Submitted Under SB950

Parent et al. (1992) Two-Year Toxicity and Carcinogenicity Study of Acrolein in Rats. J. Appl. Toxicol. 12:131-139.

Parent et al. (1992) One Year Toxicity of Orally Administered Acrolein to the Beagle Dog. J. Appl. Toxicol. 12:311-316.

Parent et al. (1992) Oncogenicity Study of Acrolein in Mice. J. Am. Coll. Toxicol. 10:647-659.

Parent et al. (1992) Reproductive Study of Acrolein on Two Generations of Rats. Fundam. Appl. Toxicol. 19:228-237.